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Review

Research Progress on Treating Spinal Cord Injury by Modulating the Phenotype of Microglia

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Abstract

Spinal cord injury (SCI) is a severe central nervous system disorder with no currently available effective treatment. Microglia are immune cells in the central nervous system that play crucial roles in the SCI occurrence, development, and recovery stages. They exhibit dynamic polarization over time and can switch between classical activation (M1) and alternative activation (M2) phenotypes to respond to environmental stimuli. The M1 phenotype is involved in initiating and sustaining inflammatory responses, while the M2 phenotype exerts anti-inflammatory effects and promotes tissue repair in damaged areas. Inhibiting M1 polarization and promoting M2 polarization have become hotspots in regulating neuroinflammation and treating SCI. This article provides a comprehensive review centered on modulating microglial polarization phenotypes for SCI treatment.

Keywords: microglia; polarization; spinal cord injury

1. Introduction

Spinal cord injury (SCI) is a severe disorder of the central nervous system (CNS), characterized by severe loss of sensory and motor function. It is often the result of falls from heights and traffic accidents [1,2]. According to reports, SCI global incidence ranges from 3.6 to 195.4 per million people, imposing a significant burden on families and societies [3]. Over the past few decades, researchers have employed various strategies to reduce neural damage and restore neural function [4]. However, there is still no universally recognized effective method for treating SCI [5,6]. The mechanism of SCI comprises two stages: primary injury and secondary injury [7]. Primary injury to the spinal cord occurs following trauma such as contusion, laceration, and compression, which directly results in cell death and instant loss of neural function. Secondary SCI involves various complex cellular and biochemical processes, including oxidative stress, inflammatory responses, cellular autophagy, and apoptosis [8]. Certain drugs are used to modulate these pathological processes and reduce or even reverse secondary SCI [9].

SCI-induced neuroinflammation involves the activation of microglia and the upregulation of pro-inflammatory cytokines, among other factors [10]. Microglia are one of the main resident cells of the spinal cord, and play a crucial role in regulating the development of neuroinflammation after SCI [11]. Microglia polarization is a microenvironment-dependent dynamic process, involved in the different stages of injury and its severity. An oversimplified and outdated view recognized that microglia polarization phenotypes are mainly classified into classical activation (M1) and alternative activation (M2). M1 microglia exacerbate neuroinflammation leading to cell death or functional impairment, which hinders SCI repair [12,13]. On the contrary, the M2 phenotype suppresses inflammation by producing anti-inflammatory factors and promoting the recovery of nearby neural function. Numerous studies have demonstrated that enhancing the M2 polarization of microglia, while inhibiting M1 polarization, can improve the efficacy of SCI treatment [14,15] (Fig. 1). Traditionally, activated M2 subtypes consist of M2a, M2b and M2c, with each having different functions in the CNS [16]. However, the classical all-or-nothing states are oversimplified and inconsistent with recently discovered phenotypes in the CNS [17]. It has been shown that microglia are not limited to a strict polarization of M1 or M2 but can display a range of intermediate phenotypes, with more subtle and dynamic functional responses that vary according to the specific environmental demands [18]. Despite this recognition, academics have yet to agree on a definition of microglial identity compared to other cell types, or on the number, dynamic nature, or definition of microglial states [19]. Although the classification of M1 and M2 is oversimplified, it helps improve our understanding of the functional state of microglia during injury progression and aids in exploring new therapeutic strategies. Thus, this classification still contributes to

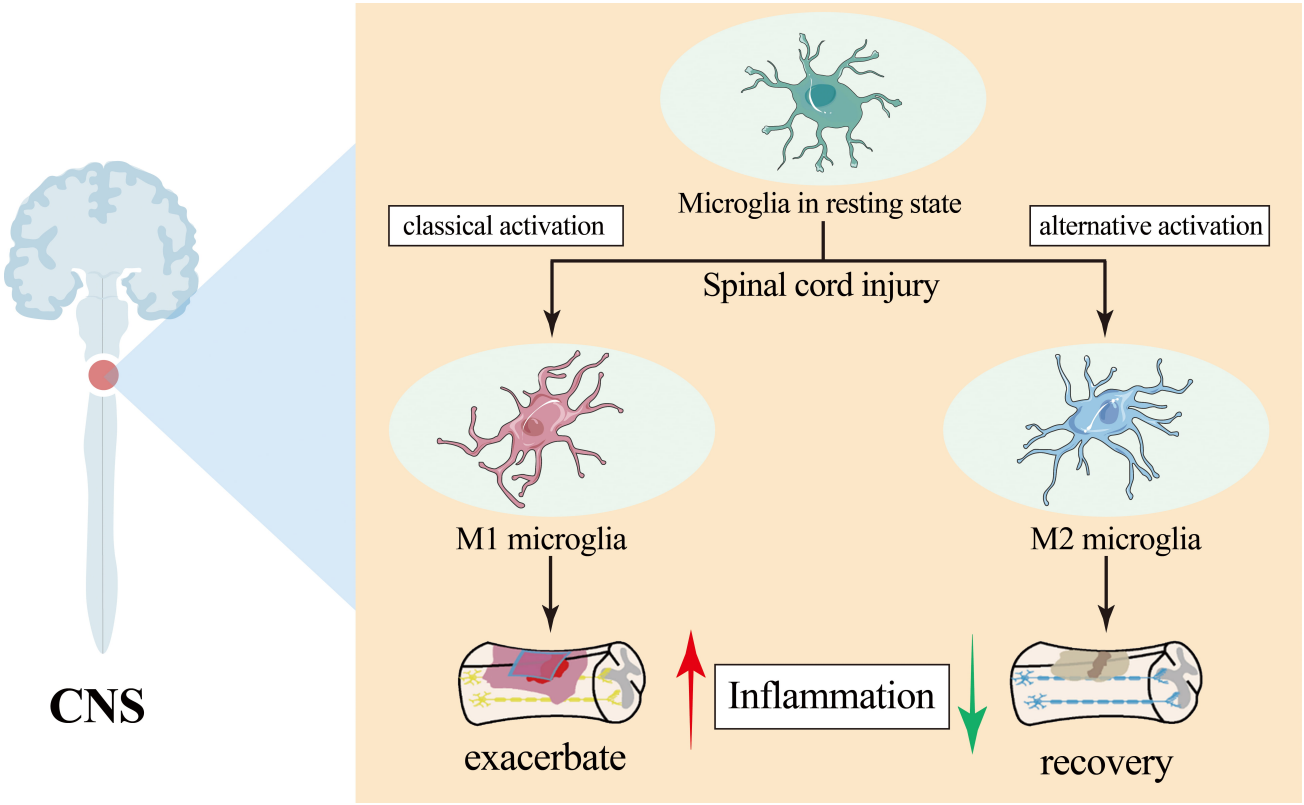

Fig. 1. Polarization and effects of microglia after spinal cord injury. Microglia polarization phenotypes are mainly classified into classical activation (M1) and alternative activation (M2). M1 microglia exacerbate neuroinflammation and the M2 microglia suppresses inflammation. CNS, central nervous system.

the understanding of microglial cell function in SCI. This article is a comprehensive review of SCI treatment methods that focus on modulating microglia polarization phenotypes.

2. Molecules

Cytokines are a class of low molecular weight proteins that play important roles in immune responses and in other physiological processes such as cell proliferation, differentiation, apoptosis, and inflammation [20]. They are primarily secreted by immune and stromal cells in response to various physiological and pathological processes [21]. Cytokines can be broadly categorized into several classes, including growth factors, interleukins (IL), interferons (IFN), tumor necrosis factors (TNF), and chemokines [22]. Imbalances in cytokines can lead to serious autoimmune diseases or inflammatory conditions, including rheumatoid arthritis and asthma [23]. Therapies targeting cytokines are effective in treating a variety of diseases [24]. Research suggests that cytokines can induce post-SCI recovery of motor function by mediating neurogenesis, neuroprotection, angiogenesis, and inflammatory responses [25] (Fig. 2).

Transforming growth factor-β1 (TGF-β1) is a multifunctional cytokine that plays a crucial role in synaptic formation, plasticity, and regulation of neurovascular units [26]. TGF-β1 treatment can decrease the polarization of M1 microglia and increase the polarization of M2 microglia [27]. In SCI mice, the administration of extracellular vesicles, released from mesenchymal stem cells (MSCs) treated with TGF-β1, increased the transition of reactive microglia from the M1 to M2 polarization state, alleviated neuroinflammation, and enhanced neuroprotection of residual cells in the acute phase [13].

Bone morphogenetic protein 7 (BMP7) has been reported to exert neuroprotective effects in different models of neurological disorders [28]. Wei *et al.* [29] investigated whether the neuroprotective function of BMP7 is associated with modulating microglia polarization. The results demonstrated that BMP7 inhibits microglia activation and promotes their conversion into M2 phenotype. These events result in the reduction of inflammatory cytokines' secretion into the microenvironment during SCI acute inflammatory phase, thereby promoting functional recovery.

IL-10 is an anti-inflammatory cytokine that can promote the recovery of neurological function after SCI [30]. Research suggests that IL-10 inhibits monocytes/macrophages inflammatory response, regulates the polarization of microglia and macrophages towards the M2 phenotype, and promotes neuronal survival [31].

IL-4 is a pleiotropic cytokine that plays a crucial role in regulating immune functions. It is considered the most

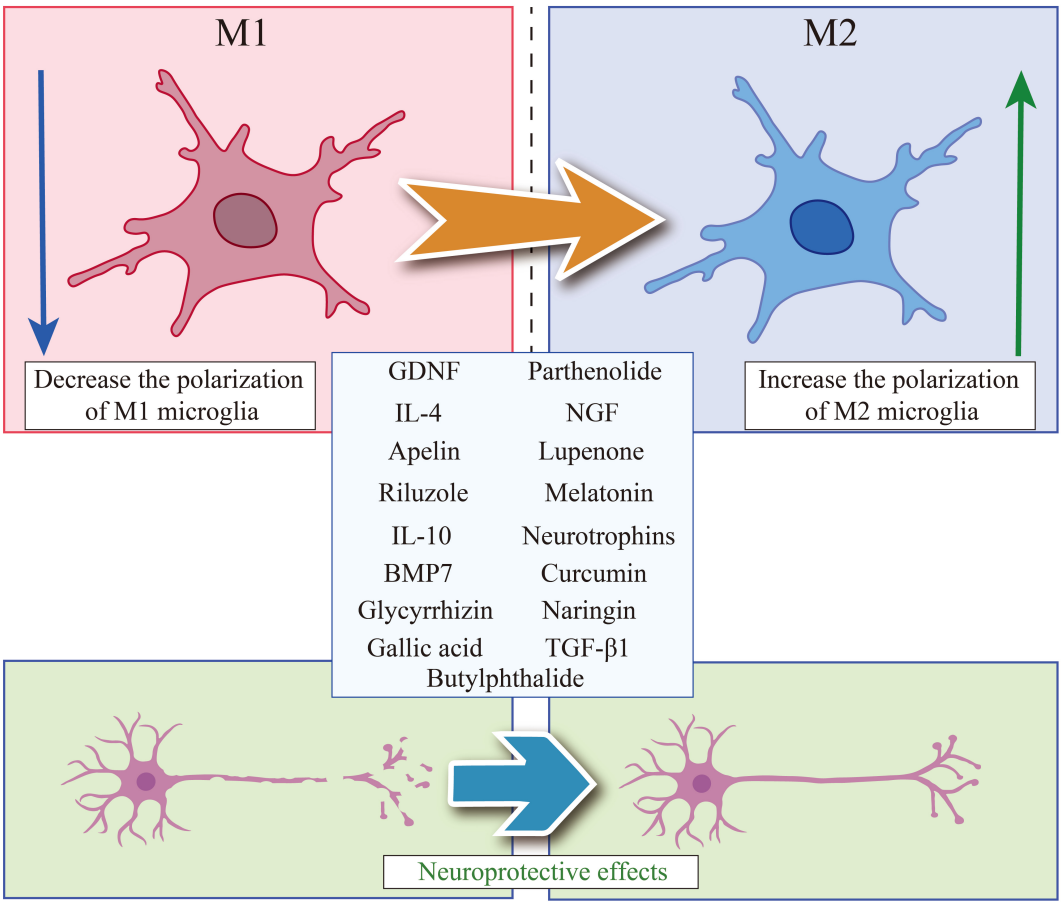

Fig. 2. Molecules that can regulate the polarization phenotype of microglia for SCI treatment. Summarized the current molecules that can inhibit polarization of M1 microglia and promote polarization of M2 microglia, which can play a neuroprotective role in the treatment of SCI. SCI, spinal cord injury; GDNF, glial cell-derived neurotrophic factor; IL-4, interleukin 4; IL-10, interleukin 10; BMP7, Bone morphogenetic protein 7; NGF, nerve growth factor; TGF-β1, transforming growth factor-β1.

potent polarizing cytokine for M2 microglia cells. IL-4 has shown beneficial activity in animal models of stroke, SCI, and multiple sclerosis [32]. Xu *et al.* [33] suggest that a 24-hour treatment with IL-4 induces M2 polarization of murine microglia, which alleviates neuroinflammation and neuronal apoptosis.

Neurotrophins are part of a unique family of polypeptide growth factors that regulate the proliferation, differentiation, survival, and death of neuronal and non-neuronal cells [34]. Many reports suggest that neurotrophins have neuroprotective functions and play a crucial role in neurological recovery after SCI [35]. In recent years, the most extensively studied neurotrophins are brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF). Komori *et al.* [36] suggest that BDNF may be associated with microglia M2 polarization. Ma *et al.* [37] developed nanoparticle scaffolds loaded with glial cell-derived neurotrophic factor (GDNF) for spinal cord repair. The results indicate that these nanoparticles can significantly promote microglia M2 polarization [37]. Moretti *et al.* [38] developed a method for treating SCI using a combination of multiple drugs. They found that NGF epidural injection reduces the activation of M1 microglia, thereby alleviating inflammation in the nervous system [38]. Akhmetzyanova *et al.* [39] found that the use of GDNF to reduce the phagocytic activity of microglia promoted the expression of a neuroprotective phenotype. Xie *et al.* [40] found that neuromuscular function improved in aged rats after treatment with GDNF, implying that GDNF has therapeutic potential for neuromuscular dysfunction in the elderly. These experimental results suggest that GDNF may be a promising therapeutic approach to promote nerve regeneration after SCI.

Neuropeptides are endogenous bioactive peptides present in various systems of the human body. They participate in neural regulation through information transmission in the nervous system and exhibit multiple effects as hormones and cytokines. Recently, melatonin and apelin have received widespread attention in the treatment of CNS diseases [41]. Melatonin is a hormone that plays a crucial role in regulating circadian rhythms. In the past, the role of

melatonin was mainly attributed to its ability to promote the initiation and maintenance of sleep. However, a large body of research suggests that melatonin also regulates microglia polarization from M1 to M2 phenotypes [42–44]. Yan *et al*. [45] investigated melatonin potential mechanisms in treating SCI. The results indicated that melatonin activates the nuclear factor erythroid 2-related factor 2 (Nrf2)/Kelch-like epichlorohydrin (ECH)-associated protein 1 (Keap1) signaling pathway and promotes microglia polarization towards the M2 phenotype [45]. Apelin is an endogenous ligand that binds the G protein-coupled receptor angiotensin-like receptor 1. According to reports, apelin has therapeutic effects on CNS diseases, such as stroke and SCI [46]. Liu *et al*. [47] found that apelin promotes the proliferation and differentiation of neural stem cells (NSCs) into neurons. Meanwhile, it can also reduce the polarization of M1-type microglia and A1-type astrocytes, promoting motor function recovery.

Recently, other natural and synthetic small molecules have been reported to improve impaired spinal cord neurological function by modulating microglia polarization [48]. Lupenone is a natural small molecule extracted from plants, such as bananas and danshen. It has been reported in numerous studies to have excellent anti-inflammatory effects [49,50]. Li *et al*. [51] evaluated lupenone therapeutic effects in a mouse model of SCI and found that it prevents SCI exacerbation by inhibiting inflammasome activation. Additionally, it enhances the transformation of pro-inflammatory M1 microglia into anti-inflammatory M2 microglia. Curcumin is a natural small molecule extracted from the rhizomes of the medicinal plant turmeric. It has been widely reported to possess a strong anti-inflammatory activity by inhibiting the production of inflammatory cytokines among others [52]. Curcumin inhibits astrocyte activation, reduces the release of pro-inflammatory cytokines, promotes the production of anti-inflammatory cytokines, and helps alleviate neuropathic pain. Moreover, it facilitates the transition of microglial phenotype from M1 to M2 [53,54]. Wu *et al*. [55] found that curcumin inhibits the activation of the nuclear factor kappa B (NF-κB) pathway by enhancing Nrf2, while also inhibiting the M1 polarization of microglia, thereby alleviating SCI. Gallic acid (GA) is a polyphenolic compound that can be fully absorbed in the body. Several reports suggested that GA possesses excellent anti-inflammatory and antioxidant stress abilities [56]. Further research found that GA inhibits the polarization of M1 microglia and promotes neuronal survival [57]. Huang *et al*. [58] confirmed that GA enhances SCI recovery in rats by modulating microglia polarization. Its potential mechanism may involve promoting the M2 polarization of microglia and the inhibition of M1 polarization. Naringin is a natural flavonoid compound, extracted from grapefruit and oranges, and renowned for its anti-inflammatory and antioxidant activities [59]. Research indicates that naringin has positive effects in alleviating nerve damage and promoting neurogenesis [60]. Further research has found that naringin can promote the transition from pro-inflammatory M1 to anti-inflammatory M2 microglia, thereby inhibiting lipopolysaccharide (LPS)-induced neuronal apoptosis [61]. Li *et al*. [62] evaluated the therapeutic effects of naringin on rats with SCI. The results showed that naringin effectively inhibits microglia activation and the expression of M1 markers in the spinal cord tissue. It also increases the expression of genes associated with M2 polarization and significantly reduces the levels of inflammatory factors. Butylphthalide is a small molecule compound isolated from celery seeds, known for its antioxidant and anti-apoptotic effects, and its ability to inhibit osteoclast formation [63]. Reports found that Butylphthalide enhances p38-dependent microglia M2 polarization and inhibits M1 polarization [64]. Glycyrrhizin is a major active component extracted from the Chinese herb, licorice. It can significantly reduce the levels of pro-inflammatory cytokines [65]. Oral administration of Glycyrrhizin reduces inflammation and improves functional recovery after traumatic SCI. The mechanism involves its inhibition of nucleotide-binding domain and leucine-rich repeat-containing proteins 3 (NLRP3) inflammasome activation and the promotion of M2 [66]. The Feverfew active ingredient, parthenolide, significantly reduces microglia M1 polarization and partially rescues LPS-induced decrease in the expression of M2 phenotype markers [67]. Riluzole is a benzothiazole derivative, commonly used to treat amyotrophic lateral sclerosis (ALS) [68]. Recent studies found that riluzole application in SCI reduces IL-6 expression levels and decreases the activation of microglia M1 phenotype expression, thereby improving functional recovery after acute SCI in rats [69,70].

In general, molecules can quickly pass through cell membranes, be absorbed by the digestive system, cause less immune response in the body, and are easy to synthesize, store, transport, and standardize, making them a promising strategy for SCI treatment. In recent years, many molecules have been reported to treat SCI by regulating the polarization of microglia. However, due to the complex pathological state during SCI, these molecules have not yet been clinically applied. In the future, safer and effective molecules need to be discovered and synthesized, and validated through clinical experiments before they can be truly applied to the treatment of SCI.

3. Gene Therapy

The Food and Drug Administration (FDA) defines gene therapy as follows: “the administration of genetic material to modify or manipulate the expression of a gene product or to alter the biological properties of living cells for therapeutic use [71]”. At present, gene therapy includes four major strategies: inhibition of abnormal transcribed RNA using microRNA, degradation of abnormal mRNA using RNA interference, decrease of mutant proteins, and DNA genome editing with methods such as clustered regu-

larly interspaced short palindromic repeats [72]. Using viral or non-viral vectors to correct or modify host genes shows promising prospects in SCI treatment. Guo *et al.* [73] found that the expression of Specific protein 1 (Sp1) in microglia increases after SCI, suggesting that Sp1 may be associated with microglia M1 polarization. In another report, they transfected synthetic siRNA-Sp1 into microglia and confirmed that its silencing inhibits microglia M1 polarization by exerting neuroprotective effects and promoting functional recovery after SCI. 5-Hydroxytryptamine receptor 2B (Htr2b) is one of the receptors of serotonin (5-HT) and is generally thought to promote inflammation. Chen *et al.* [74] applied shRNA lentivirus targeting of Htr2b and the results showed that its knockdown inhibits microglia M1 polarization and neuroinflammation after SCI. TNF-α induced protein 3-interacting protein interacting protein 2 (TNIP2), is a negative regulator of NF-κB signaling. TNIP2 overexpression inhibits microglia M1 polarization and proinflammatory cytokine production [75]. Poly (adenosine diphosphate [ADP]-ribose) polymerase family member 14 (PARP14) has been reported to promote post-stroke functional recovery. Xu *et al.* [33] found that PARP14 overexpression promotes M2 polarization, thereby improving neurological function recovery after SCI. Fan *et al.* [76] developed an efficient delivery system for transporting miRNA-124-3p to the center of the SCI, where it released abundant microRNAs to elicit axon sprouting and rehabilitation of the inflammatory microenvironment. The delivery system has also been proven to promote neuronal axon growth and microglia M2 polarization.

The above studies suggest that regulating the polarization of microglia through gene therapy is a potent method for treating SCI. The safety of several viral vectors for gene therapy has been widely reported after decades of clinical studies. Although cell therapy for SCI has entered the clinical trial stage, research on gene therapy for SCI has not yet reached the clinical stage [77]. Improving the transfection efficiency of gene vectors, localizing and regulating gene delivery, and continuing preclinical research to confirm the safety and effectiveness of gene therapy are future directions.

4. Exosomes

In all biological systems, cells secrete exosomes in physiological and pathological states. Exosomes are tiny vesicles encapsulated by membranes that are essential in the process of communication between cells [78]. Exosomes can influence the differentiation of neuroglial cells and regulate neuroinflammation. This process triggers the release of cytokines and inflammatory mediators, enhances resistance to cell apoptosis, and exhibits neuroprotective effects [79]. Luo *et al.* [80] found that Adipose-derived MSCs exosomes inhibit the expression of inflammatory factors in the spinal cord tissues and M1 microglia, promote M2 microglia, and activate the Nrf2/heme oxygenase-1 (HO-1) pathway. Xue *et al.* [81] confirmed that injection of bone marrow mesenchymal stem cell (BMSCs) exosomes suppresses microglia M1 polarization-mediated inflammation. Ren *et al.* [82] evaluated the regulation of inflammation of Schwann cell-derived exosomes and demonstrated that these exosomes attenuate the inflammation by suppressing M1 polarization and stimulating M2 polarization. Fan *et al.* [83] developed an exosomes-loaded electroconductive hydrogel and showed its capacity to modulate microglia M2 polarization via the NF-κB pathway.

Exosomes are crucial mediators of intercellular communication, widely present in body fluids, and play a role in various pathological processes in the body. Compared to cells, exosomes have a smaller nanoscale structure and good biocompatibility. They can pass the blood-brain barrier and hold promise as drug carriers [84]. However, several issues need addressing when using extracellular vesicles for clinically treating SCI, such as developing standardized methods for separating high-purity extracellular vesicles, identifying optimal sources of extracellular vesicles, and establishing protocols for storage and transportation. Further research is still needed to address these issues and pave the way for the clinical application of extracellular vesicles in the treatment of SCI.

5. Cell Therapy

The use of autologous or allogeneic cell transplantation to repair SCI tissue damage is a potential treatment [85]. Transplanting NSCs, BMSCs, and olfactory ensheathing cells (OECs) have been studied as potential SCI therapeutic methods [86,87]. Transplanted NSCs reduced the number of infiltrated immune cells and biased microglia towards a regenerative M2 phenotype, suggesting a long-term impact on the functional recovery of SCI rats [88]. Guo *et al.* [89] demonstrated that transplanted olfactory OECs effectively enhance neural survival and axonal outgrowth. The therapeutic effect is mainly attributed to the anti-inflammatory activity of OECs which modulates the polarization of microglia from the M1 to M2 phenotype. Pang *et al.* [90] found that MSCs transplantation after SCI may induce a shift in the phenotype of microglia/macrophages from M1 to M2, providing an anti-inflammatory and reparative microenvironment for motor recovery.

Cell therapy is considered the most promising strategy for treating SCI. Cells transplanted into the region of SCI regulate inflammatory responses or promote axonal regeneration and nerve repair through multidirectional differentiation and secretion of cytokines or neurotrophic factors [91]. Despite promising progress in research related to cell therapy, many challenges remain. These include determining the optimal number of cell transplants, identifying the optimal time window, addressing concerns about tu-

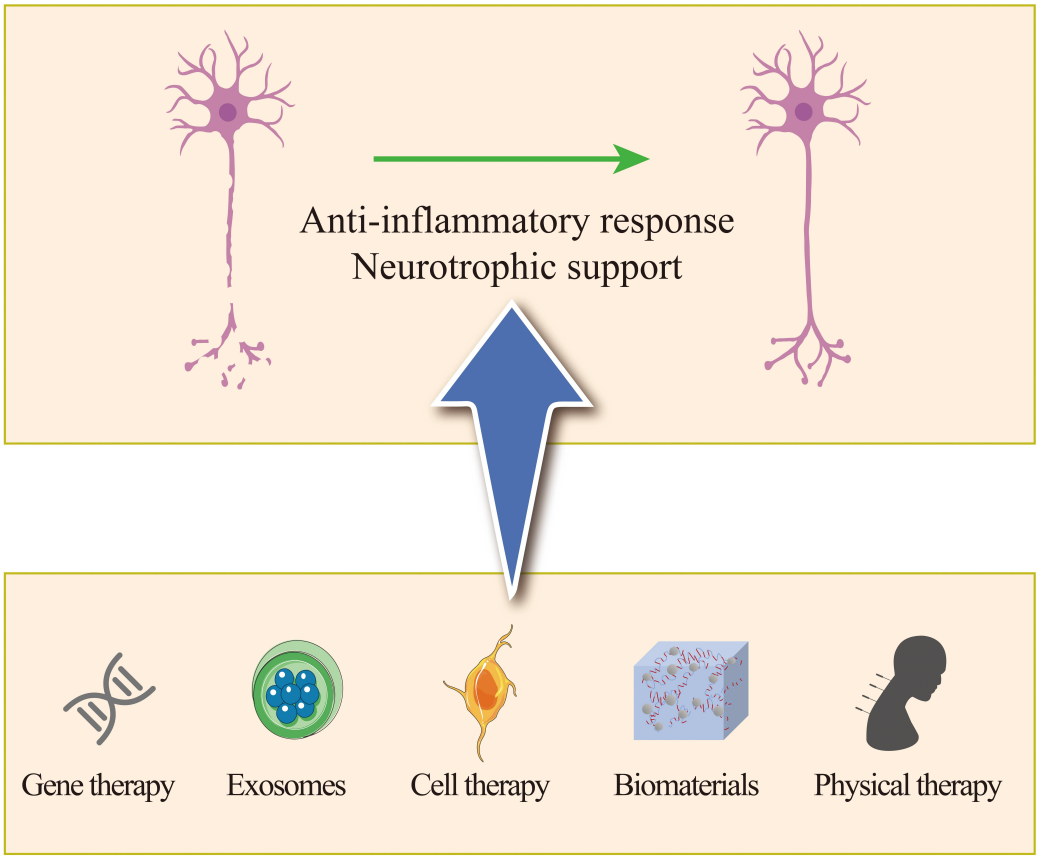

Fig. 3. Introduce the current methods for treating spinal cord injury. The methods for treating spinal cord injury are mainly divided into 5 categories, including gene therapy, exosomes, cell therapy, biomaterials, and physical therapy, all of which can inhibit inflammatory reactions and provide neuroprotective effects.

morigenicity, and improving the low survival rate of transplanted cells. Therefore, cell therapy still requires substantial data support before it can be applied on a large scale in clinical settings.

6. Biomaterials

In recent years, regenerative medicine based on biomaterial has rapidly developed, and biomedical materials-based strategies for SCI repair have also received widespread attention [92,93]. Biomedical materials have promising prospects in regulating microglial polarization to repair spinal cord injuries [94]. They can be categorized as nanoparticles, gels, and scaffolds and their functions involve transmitting signaling molecules, and encapsulated cells [95].

Nanoparticles are the smallest carriers and are typically employed for transporting small molecule drugs. These nanoparticles extend the residence time of drugs in the lesion area, enhance the local effective concentration, and concurrently reduce the systemic impact of drugs on the body [96]. Gopalakrishnan *et al.* [97] developed nanoparticles incorporating carbohydrate antigens and showed that these nanoparticles activate resting human microglia and polarize them toward a putative M2 state. Zhou *et al.* [98] used gold nanoclusters loaded with berberine to reduce inflammation by inhibiting the activation of M1 phenotype microglia, which simultaneously inhibited neuronal apoptosis after SCI.

Hydrogel is a three-dimensional polymer material with a widely hydrophilic structure, capable of providing a suitable aqueous environment for cells and promoting cell proliferation [99]. A biocompatible hydrogel loaded with fat extract was used to treat a model of spinal cord contusion in mice. The composite promoted the polarization of macrophages from an inflammatory M1 phenotype to an anti-inflammatory M2 phenotype [100]. Yu *et al.* [101] developed a fibronectin hydrogel containing lycium barbarum oligosaccharide and nasal mucosa-derived MSCs for SCI restoration by leveraging the inflammatory licensing effect and microglia M2 polarization.

At present, some biomaterials used for the treatment of SCI have entered the clinical trial stage and have demonstrated evidence of safety and effectiveness [102]. Biomaterials can serve as carriers for targeted delivery and sustained release of drugs in SCI areas, mimicking the soft tissue microenvironment to effectively guide and support the repair process. They can also be combined with cell therapy to create a conducive environment for transplanted

Table 1. Summary of recent research on regulating microglial polarization in the treatment of spinal cord injury.

Category	Type of research	Model	Type of injury	Treatment	Effect	Reference
Molecules	Preclinical study	Spinal cord injury in mice	Contusion	Administration of extracellular vesicles, released from MSCs treated with TGF-β1 via the tail vein	Increased the transition of reactive microglia from M1 polarization to M2 polarization, alleviated neuroinflammation, and enhanced the neuroprotective effect of residual cells in the acute phase.	[13]
	Preclinical study	Spinal cord injury in rats	Contusion	Local injection of recombinant human BMP7	Suppressed the viability of microglia cells and increased the proportion with the M2 phenotype, thus reducd neuron loss in the injured spinal cord and promoted functional recovery after SCI.	[29]
	Preclinical study	Spinal cord injury in mice	Complete transection	Injection of IL-10-releasing hydrogel	Promoted the M2 macrophage/microglia phenotype, and led to neural regeneration and axon growth.	[31]
	Preclinical study	Spinal cord injury in mice/Experiments on microglia *In vitro*	Contusion/Treatment of microglia using IL-4 *In vitro*	Injection of PARP14 shRNA-carrying lentivirus to silence PARP14 expression/Transfected with an adenovirus PARP14 overexpression vector	PARP14 knockdown activated microglia in the spinal cord and promoted a shift from M2-polarized to M1-polarized/IL-4 treatment promoted M2 polarisation in microglia. In addition, PARP14 overexpression made microglia more prone to M2 polarization.	[33]
	Preclinical study	Spinal cord injury in mice	Hemisection	Transplantion of GDNF-loaded nanoparticles	GDNF-loaded nanoparticles promoted microglia M2 polarization, thereby inhibited inflammatory response at the injury site.	[37]
	Preclinical study	Spinal cord injury in rats	Contusion	Epidural injection of nanomedicines loaded with NGF	A strong anti-inflammatory effect was observed in the short term with a reduction of type M1 microglia.	[38]
	Preclinical study	Spinal cord injury in mice	Contusion	Intraperitoneal injection of melatonin	Melatonin activated the Nrf2/Keap1 signaling pathway and promoted microglia polarization towards the M2 phenotyp.	[45]
	Preclinical study	Spinal cord injury in rats	Complete transection	Transplantion of induced pluripotent stem cells (iPSCs) infected with lentivirus bearing an apelin expression vector	Transplantation of transfected iPSCs *in situ* immediately after SCI reduced polarization of M1 microglia, facilitated recovery of motor function.	[47]
	Preclinical study	Spinal cord injury in mice	Contusion	Intraperitoneal injection of lupenone	Lupenone enhanced the conversion of proinflammatory M1 microglial cells into anti-inflammatory M2 microglial cells, and protect against spinal cord injury by inhibiting inflammasomes.	[51]
	Preclinical study	Spinal cord injury in rats	Ischemia-reperfusion injury	Intraperitoneal injection of curcumin	Curcumin restrained microglia M1 activation and neuroinflammation in spinal cord tissues.	[55]
Molecules	Preclinical study	Spinal cord injury in rats	Contusion	Intraperitoneal injection of GA	GA promoted recovery in SCI rats by promoting microglia M2 polarisation and inhibiting M1 polarisation.	[58]

Table 1. Continued.

Category	Type of research	Model	Type of injury	Treatment	Effect	Reference
	Preclinical study	Spinal cord injury in rats	Contusion	Gavage administration of naringin	Naringin effectively inhibited microglial activation and expression of M1 markers in spinal cord tissues. It also elevated M2 polarization-related gene expression and significantly lowered the levels of inflammatory factors.	[62]
	Preclinical study	Spinal cord injury in mice	lateral compression	Injection of butylphthalide through the caudal vein	Treatment with butylphthalide could reduce pro-inflammatory cytokine release after SCI and could facilitate macrophage/microglia M2 polarization and inhibit M1 polarization after SCI.	[64]
	Preclinical study	Spinal cord injury in rats	Contusion	Oral treatment with glycyrrhizin	Oral treatment with glycyrrhizin promoted microglial M2 polarization and improved functional recovery after traumatic SCI.	[66]
	Preclinical study	Spinal cord injury in mice	Contusion	Intraperitoneal injection of parthenolide	Parthenolide promoted axonal regeneration, increased myelin reconstitution, and facilitated shift from M1 to M2 polarization of microglia.	[67]
	Preclinical study	Spinal cord injury in rats	Contusion	Intraperitoneal injection of riluzole	Riluzole applied in a single dose immediately post-SCI reduced the destruction of neurons, and reduced the activation of microglia M1 expression at day 1 post-SCI.	[69]
	Preclinical study	Spinal cord injury in rats	Contusion	Intraperitoneal injection of riluzole	Riluzole upregulated the mRNA levels of M2 markers, but downregulated that of M1 markers.	[70]
Gene therapy	Preclinical study	Spinal cord injury in rats/Experiments on microglia *In vitro*	Contusion/Lipopolysaccharide treatment	Detection of SP1 expression in injured spinal cord/Silencing SP1 in microglia using siRNA-Sp1	SP1 was highly expressed in SCI rats. Sp1 knockdown restrained M1 polarization of microglia and its associated inflammation.	[73]
	Preclinical study	Spinal cord injury in rats/Experiments on microglia *In vitro*	Contusion/Lipopolysaccharide treatment	Detection of Htr2b expression in injured spinal cord/Inhibition of Htr2b by shRNA-Htr2b	Htr2b was highly expressed in SCI rats. In addition, inhibition of Htr2b reduced M1 polarization of microglia.	[74]
Gene therapy	Preclinical study	Spinal cord injury in rats/Experiments on microglia *In vitro*	Contusion/Lipopolysaccharide treatment	Detection of TNIP2 expression in injured spinal cord/Transfection of microglia with TNIP2-overexpressing lentiviral	TNIP2 expression was increased during SCI in rats and that overexpression of TNIP2 inhibited M1 polarization and pro-inflammatory cytokine production in microglia.	[75]
	Preclinical study	Spinal cord injury in mice	Contusion	Injection of PARP14 shRNA-carrying lentivirus	PARP14 knockdown promoted microglia M1 polarization.	[33]
Exosomes	Preclinical study	Spinal cord injury in rats	Contusion	Injection of adipose-derived MSCs exosomes via tail veins	Adipose-derived MSCs exosomes inhibited the expression of both inflammatory factors in the spinal cord tissues and M1 microglia, promoted the expression of M2 microglia.	[80]
	Preclinical study	Spinal cord injury in rats	Contusion	Injection of miR-216a-5p-overexpressed BMSCs-exosomes via tail veins	The injection of miR-216a-5p-overexpressed BMSCs-exosomes improved locomotor performance, while inhibiting neuronal apoptosis and microglia M1 polarization.	[81]

Table 1. Continued.

Category	Type of research	Model	Type of injury	Treatment	Effect	Reference
	Preclinical study	Spinal cord injury in rats	Contusion	Injection of Schwann cell-derived exosomes via tail veins	Schwann cell-derived exosomes could attenuate the inflammation in SCI rats by suppressing M1 polarization and stimulating M2 polarization.	[82]
	Preclinical study	Spinal cord injury in mice	Hemisection	Transplantion of hydrogels loaded with BMSC-exosomes	Exosomes-loaded hydrogels modulate microglial M2 polarization.	[83]
Cell therapy	Preclinical study	Spinal cord injury in rats	Complete transection	Transplantion of NSCs	NSCs reduced the number of infiltrated immune cells, biased microglia towards a regenerative M2 phenotype.	[88]
	Preclinical study	Spinal cord injury in rats	Contusion	Transplantion of OECs	OECs modulated microglial polarization from the M1 to M2 phenotype.	[89]
	Preclinical study	Spinal cord injury in rats	Contusion	Transplantion of MSCs	MSCs increased numbers of M2 microglia and decreased numbers of M1 microglia.	[90]
Biomaterials	Preclinical study	Experiments on microglia *In vitro*	None	Treatment of microglia with nanoparticles incorporating carbohydrate antigens	Resting microglia exposed to nanoparticles can be activated and polarized toward M2 state.	[97]
Biomaterials	Preclinical study	Spinal cord injury in rats	Contusion	Injection of gold nanoclusters loaded with berberine via tail veins	Nanoclusters reduced M1 protein marker CD86, increased M2 protein marker CD206, reduced inflammation and apoptotic cytokines.	[98]
	Preclinical study	Spinal cord injury in mice	Contusion	Treatment with a biocompatible hydrogel loaded with fat extract	The composite promoted the polarization of microglia from an inflammatory M1 phenotype to an anti-inflammatory M2 phenotype.	[100]
	Preclinical study	Spinal cord injury in rats	Complete transection	Treatment with fibronectin hydrogel containing lycium barbarum oligosaccharide and nasal mucosa-derived MSCs	The hydrogel possesses a synergistic effect on M2 polarization of microglia.	[101]
Physical therapy	Preclinical study	Spinal cord injury in rats	Clip-compression	Transcranial direct current stimulation	Transcranial direct current stimulation reduced the proportion of the M1 phenotype of microglia and increase the proportion of the M2 phenotype.	[106]
	Preclinical study	Spinal cord injury in rats	Complete transection	Electroacupuncture	Electroacupuncture improved BBB scores, inhibited the proportion of M1 microglia.	[107]

TGF-β1, transforming growth factor-β1; MSCs, mesenchymal stem cells; IL, interleukin; BMP7, Bone morphogenetic protein 7; SCI, Spinal cord injury; PARP14, Poly (adenosine diphosphate [ADP]-ribose) polymerase family member 14; GDNF, glial cell-derived neurotrophic factor; GA, Gallic acid; SP1, Specific protein 1; Htr2b, 5-Hydroxytryptamine receptor 2B; TNIP2, TNF-α induced protein 3-interacting protein interacting protein 2; TNF, tumor necrosis factor; BMSCs, bone marrow mesenchymal stem cell; OECs, olfactory ensheathing cells; NSCs, neural stem cells; Nrf2, nuclear factor erythroid 2-related factor 2; Keap1, Kelch-like epichlorohydrin (ECH)-associated protein 1; BBB, blood brain barrier; NGF, nerve growth factor.

cells, promoting their proliferation and differentiation. Despite the promising prospects of combining biomaterials with other therapies for SCI treatment, there is still insufficient clinical research in this area, necessitating further studies to confirm its effectiveness.

7. Physical Therapy

Physiotherapy is a practical treatment for people with spinal cord injuries. Acupuncture, current stimulation, and other treatments have been widely reported to significantly improve motor function in SCI patients [103,104]. Some physical therapies have also been proven to alleviate neuroinflammation and secondary injury after SCI [105]. Tan *et al*. [106] confirmed that transcranial direct current stimulation reduces the proportion of microglia M1 phenotype and increases the proportion of the M2 phenotype. Zhao *et al*. [107] found that electroacupuncture improves blood brain barrier (BBB) scores, decreases the proportion of M1 macrophages, TNF-α, IL-1β and IL-6 levels, and enhances IL-10 levels and the proportion of M2 microglia.

In clinical practice, physical therapy is often applied to SCI patients once their condition has stabilised with the main aim of promoting the recovery of motor function and preventing complications. Physical therapy represents the most common therapeutic strategy for spinal cord injuries, due to its non-invasive nature and feasibility [1]. The repair of SCI is a complex pathophysiological process. Over the past few decades, numerous research efforts have been conducted, achieving certain success through strategies such as gene therapy, biomaterials, and exosomes to modulate the phenotype of microglia and promote SCI repair (Fig. 3). However, due to the complexity of SCI repair, no ideal repair strategy has yet been found to fully repair and regenerate SCI. Hence, the combination and synergy of physiotherapy with other therapies may represent a promising avenue for future research in SCI therapy [108,109].

8. Conclusions and Perspectives

SCI is a severe condition that can lead to paralysis of the limbs, respiratory system impairment, and restricted mobility, imposing significant physical, psychological, and economic burdens on patients. SCI current clinical treatments, including surgical decompression, drug therapy, and physical rehabilitation, have limited effectiveness. SCI-associated inflammation can exacerbate tissue damage and functional loss, leading to secondary injury. Studies indicate that reducing inflammation and immune cell infiltration in the damaged CNS may improve neuronal regeneration. M1 microglia secrete inflammatory factors at the injury site, exacerbating spinal cord damage. In contrast, M2 macrophages secrete IL-4, IL-10, and neurotrophic factors to suppress inflammation and neuronal apoptosis. Increasing evidence suggests that inhibiting microglia M1 polarization to suppress the release of pro-inflammatory mediators may have neuroprotective effects. Meanwhile, M2 microglia can produce anti-inflammatory cytokines and promote angiogenesis, facilitating neural recovery in injured spinal cords and inhibiting neuronal death. We have summarized recent research on regulating microglia polarization for SCI treatment (Table 1, Ref. [13,29,31,33,37,38,45,47,51,55,58,62,64,66,67,69,70,73–75,80–83,88–90,97,98,100,101,106,107]). Despite the several studies aimed at elucidating the regulatory mechanisms of microglia, there is currently no clinically effective method to promote microglia transition into a neuroprotective phenotype to stimulate neural regeneration. Clinical trials have been carried out to test advanced treatments such as biomaterial, cell therapy, and physical therapy. However, it is still challenging to remodel neurological function due to the complex pathological process of SCI. It is difficult to translate animal studies to clinical practice owing to species differences. In the future, more research is needed to further analyze the pathological mechanisms of SCI to explore new therapeutic targets and approaches. Modulation of microglial function is an emerging and promising treatment strategy for SCI recovery. In conclusion, targeted modulation of microglial polarization holds promise as a future therapeutic approach for SCI aimed at promoting neural regeneration and improving functional recovery.

Author Contributions

QY and ZC conducted a literature review, wrote the manuscript and designed the figures and tables. XL, SL, PL, YR and JL conducted literature searches and assisted in writing. XH and WL was responsible for the conceptualization, supervision and reviewing the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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